Re: United States Patent Application No. 09/811,123
Filed: March 16, 2001
Title: Methods of Treatment Using Anti-ErbB Antibody-Maytansinoid Conjugates
Inventors: ERICKSON et al.

DECLARATION

I, Mark X. Sliwkowski of San Carlos, California, do declare that:

1. I am an inventor of United States Patent Application Serial No. 09/811,123 of Erickson et al.

2. I have over 14 years of experience in cancer biology research at Genentech, Inc., including research on drugs directed against the human epidermal growth factor receptor family (also known as the HER or ErbB family). Two of these drugs, HERCEPTIN® (Trastuzumab) and Tarceva® (erlotinib) have received U.S. Food and Drug Administration approval. I am currently employed as Director, Staff Scientist: Translational Oncology at Genentech, Inc., and have previously served as a Staff Scientist, at Triton Biosciences, Inc. (Berlex Biosciences, Inc.) from 1985-1991, and as a Staff Fellow at National Institutes of Health, National Heart, Lung and Blood Institute, Laboratory of Biochemistry (1982-1985). I received a Ph.D. degree in Biochemistry from North Carolina State University in 1981. I received the 2005 Pharma Achievement Award as Industry Scientist of the Year. I am an author on many published scientific articles related to my work, have published many abstracts and presented my work at numerous scientific meetings, and am an inventor on several patents. Some of my published articles related to breast cancer and the ErbB receptor are listed in Appendix 1 herein.

3. I have read and am familiar with the specification and pending claims of the United States patent application entitled METHODS OF TREATMENT USING ANTI-ErbB ANTIBODY-MAYTANSINOID CONJUGATES, Serial No. 09/811,123 to Erickson et al.

4. As the Research Project Team Leader and then later the Early Development Team Leader at Genentech, I was responsible for planning, gathering and coordinating the scientific work related to antibody drug conjugates, including the anti-ErbB antibody-maytansinoid conjugate known as trastuzumab-SMCC-DM1. I have worked with other scientists and research associates who are engaged in research in these and in related fields and have observed their level of skill. Based on my education, training, and extensive experience as a scientist in academic and industrial laboratories, I have direct knowledge of the skills and techniques available to one of ordinary skill in the art in the fields related to antibody treatments for cancer, and to antibody-drug conjugates.

5. It is my considered opinion, based on my experience as a research scientist, that the level of skill in the relevant art is very high, most researchers in the area of antibody conjugation research having advanced degrees, and most having extensive laboratory training and experience.

6. I have read and am familiar with the specification and pending claims of the present application. Based on my training and experience, I am familiar with scientific fields related to antibody treatments for cancer, and to antibody-drug conjugates.

7. I note that some of the methods for identifying ErbB-expressing cells to which anti-ErbB antibodies bind were disclosed in the specification of the subject application at pages 58-60 (paragraphs [0211] - [0221] US 2002/0001587). Methods for identifying the target population of patients with ErbB overexpressing tumors were disclosed in the specification of the subject application. For example, the specification at page 60 (paragraph [0224] US 2002/0001587)discusses identification of such patients by an ordinary skilled physician.

8. I am aware of experimental results providing measurements of expression of ErbB and identifying tumor cells which respond to anti-ErbB antibodies. The dynamic range of ErbB2 or HER2 overexpression spans about two orders of magnitude. Normal breast epithelial cells express HER2 at ~10,000 receptors per cell. In contrast tumors that contain HER2 gene amplification may have 1,000,000 HER2 receptors per cell. Using a panel of human breast cancer cell lines, G.D. Lewis et al. (1993) Cancer Immunol Immunother 37:255 demonstrated that the cytostatic effects of HER2 antibodies correlated with HER2 expression level. A threshold of HER2 overexpression was required in order to see cytostasis. This threshold is now thought to be ~100,000 HER2 receptors per cell.

9. As noted in the aforementioned Lewis publication, growth of the breast cancer cell lines SK-BR-3, BT474, MDA-MB-453, MDA-MB-361 is inhibited by certain HER2 antibodies. Thus, these cell lines are some of the cell lines which respond to anti-ErbB antibodies (population A).

10. Many such cancer cell lines which respond to anti-ErbB antibodies (population A) exist and have been studied as xenograft tumors in mice, such as Calu3 xenograft tumors in SCID-beige mice (see attached graph of mean tumor volume progression after IV dosing, Appendix 2, Figure 1). The Calu3 tumors respond well to the anti-ErbB antibody trastuzumab (HERCEPTIN®) with partial remission of 8 of 9 tumor-bearing mice after dosing at 31 mg/kg every 3 weeks. The effects of treatment with naked, unconjugated anti-ErbB antibodies are cytostasis, but not cell death or cytotoxicity.

11. Some population A cell lines or tumors have also been treated with anti-ErbB antibody-maytansinoid conjugates. Complete remission of 8 of 9 Calu3 xenograft tumor bearing mice was observed in the groups dosed with 500 and 1500 $\mu g/m^2$ of trastuzumab-SMCC-DM1 every three weeks (see Appendix 2, Figure 1). In mice, a dose

of 500 μg/m^2 is equal to 165 μg/kg. The effect of treatment of Calu3 xenograft tumor bearing SCID-beige mice resulted in cell death, i.e. cytotoxicity.

12. These experiments can be described as follows. Briefly, Calu3 cells are placed subcutaneously in the flank of immune deficient mice. Depending on the cell line and other conditions, palpable tumors will generally appear within 21 days or so. A tumor of 100 mm^3 is generally thought to be 'established'. Once a sufficient number of mice harbor these established tumors, they are randomized to various number of treatment groups. A typical experiment may have 8 treatment groups with 8-10 animals per group. Randomization ensures that each group has on average the same tumor volume. One or more groups are generally assigned as 'control' groups to internally calibrate therapeutic effects of test agents. Examples of controls might be a vehicle or excipient or a non-specific isotype control antibody. A treatment dose and regimen is pre-determined for each experiment and experimental agent. The therapeutic effect of the test agent is assessed by measuring tumor volumes on a weekly or more frequent basis. The toxicity of the test agent is assessed by measuring body weight, specific stress or damage enzymes in the serum of these animals, cellular blood components and general health of the animals.

13. In addition, we have conducted many experiments which identify cells or tumors which do not respond, or respond only poorly, to anti-ErbB antibodies (population B). This is the basis of the entire research program. Cell lines for *in vitro* proliferation studies and cell lines for xenograft tumor models were selected to set a very high bar for potency, i.e. where the cells do not respond, or respond only poorly, to unconjugated anti-ErbB antibodies.

14. For example, another cell line, BT474E1, in a xenograft tumor in beige nude mice, responded poorly to dosing of trastuzumab at 15 mg/kg every 3 weeks (Appendix 2). Tumors progressed in mice treated with trastuzumab at approximately the same rate as mice treated with the buffer vehicle (placebo).

15. We have also treated cell lines, or tumors of cells which do not respond, or respond only poorly, to anti-ErbB antibodies (population B) with anti-ErbB antibody-Maytansinoid conjugates. These experiments were as described for the Calu3 tumors, except that BT474EI cells are used. The effects of treating some population B xenograft tumor animals and cancer cell lines with anti-ErbB antibody-maytansinoid conjugates are striking. For example, partial and complete remission of BT474E1 xenograft tumor bearing mice was observed in the groups dosed with 150, 500, and 750 μg/m2 of trastuzumab-SMCC-DM1 every three weeks (see graph included here). A dose dependent effect was observed. The effect of treatment of BT474E1 xenograft tumor bearing mice resulted in cell death, i.e. cytotoxicity.

16. There are differences in the responses to anti-ErbB antibody-maytansinoid conjugates between cells, cell lines or tumors of population A and population B, in that all the ErbB expressing cells respond to anti-ErbB antibody-maytansinoid conjugates, but only some ErbB expressing cells respond to unconjugated anti-ErbB antibodies.

17. Based on my experience in conducting research and working with scientists in cancer research over more than fourteen years, and based on my familiarity with the specification of the present application, I believe that the specification provides sufficiently detailed instructions so as to allow a scientist of ordinary skill to determine cells and tumors which overexpress ErbB2 receptor and which do not respond, or respond poorly to treatment with an anti-ErbB2 antibody, and how to treat such cells or tumors with anti-ErbB antibody-maytansinoid conjugates.

18. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true. I declare that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patent issuing from the application thereon.

Date: Sept 2, 2005

Mark X. Sliwkowski, Ph.D.
San Carlos, California

Appendix 1:

Franklin MC, Carey KD, Vajdos FF, Leahy DJ, de Vos AM, Sliwkowski MX. Insights into ErbB signaling from the structure of the ErbB2-pertuzumab complex. *Cancer Cell* 2004 Apr; 5(4): 317-28.

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Lewis GD, Lofgren JA, McMurtrey AE, Nuijens A, Fendly BM, Bauer KD, Sliwkowski MX. Growth regulation of human breast and ovarian tumor cells by heregulin: Evidence for the requirement of ErbB2 as a critical component in mediating heregulin responsiveness. *Cancer Res* 1996 Mar 15; 56(6): 1457-65.

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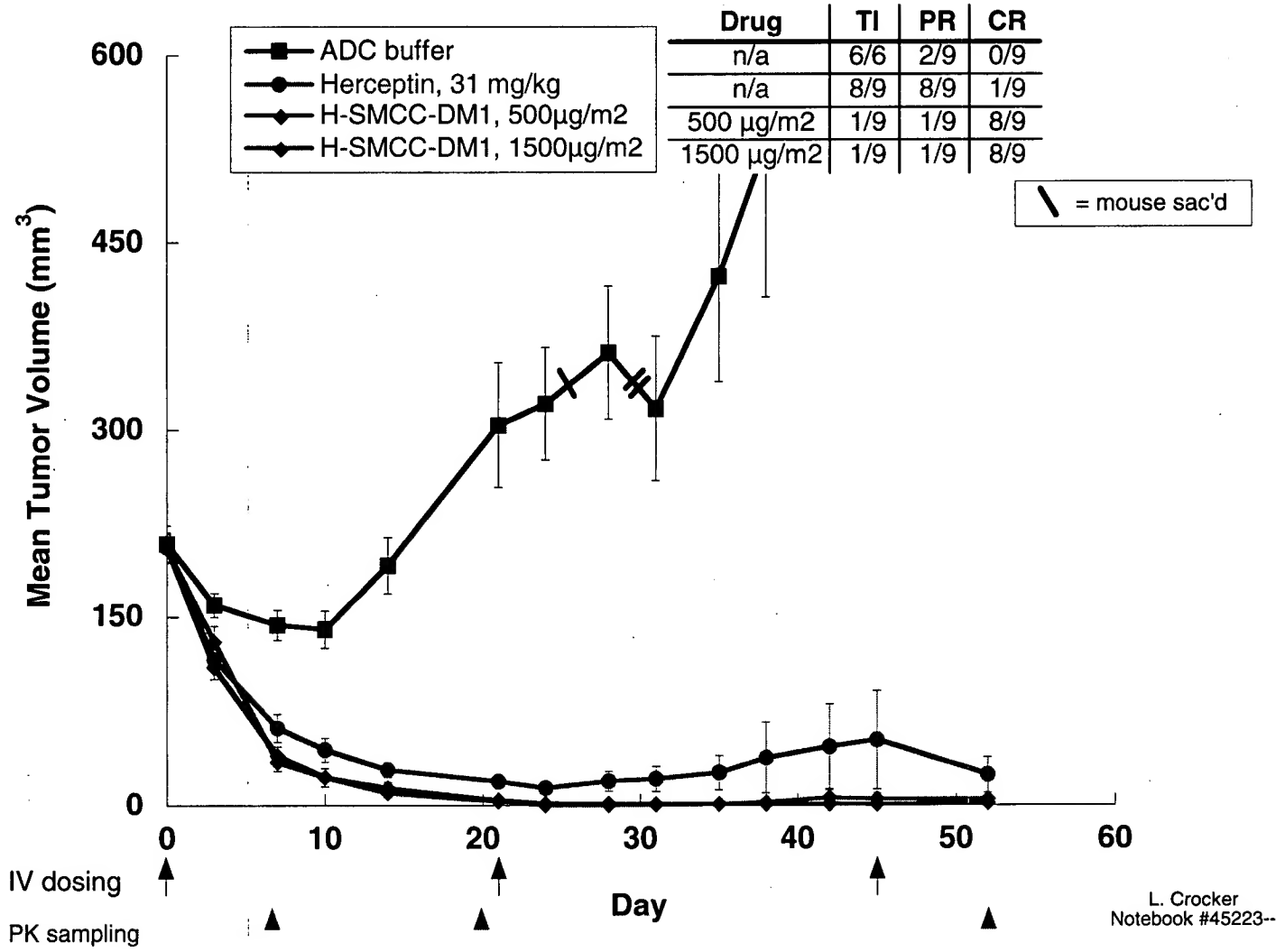
Sliwkowski MX, Schaefer G, Akita RW, Lofgren JA, Fitzpatrick VD, Nuijens A, Fendly BM, Cerione RA, Vandlen RL, Carraway KL 3rd. Coexpression of erbB2 and erbB3 proteins reconstitutes a high affinity receptor for heregulin. *J Biol Chem* 1994 May 20; 269(20): 14661-5.

Holmes WE, Sliwkowski MX, Akita RW, Henzel WJ, Lee J, Park JW, Yansura D, Abadi N, Raab H, Lewis GD, et al. Identification of heregulin, a specific activator of p185erbB2. *Science* 1992 May 22; 256(5060): 1205-10.

04-0061A: Efficacy of Herceptin-SMCC-DM1 vs. Calu3 Xenograft Tumors in SCID-beige Mice (10 million cells/mouse)

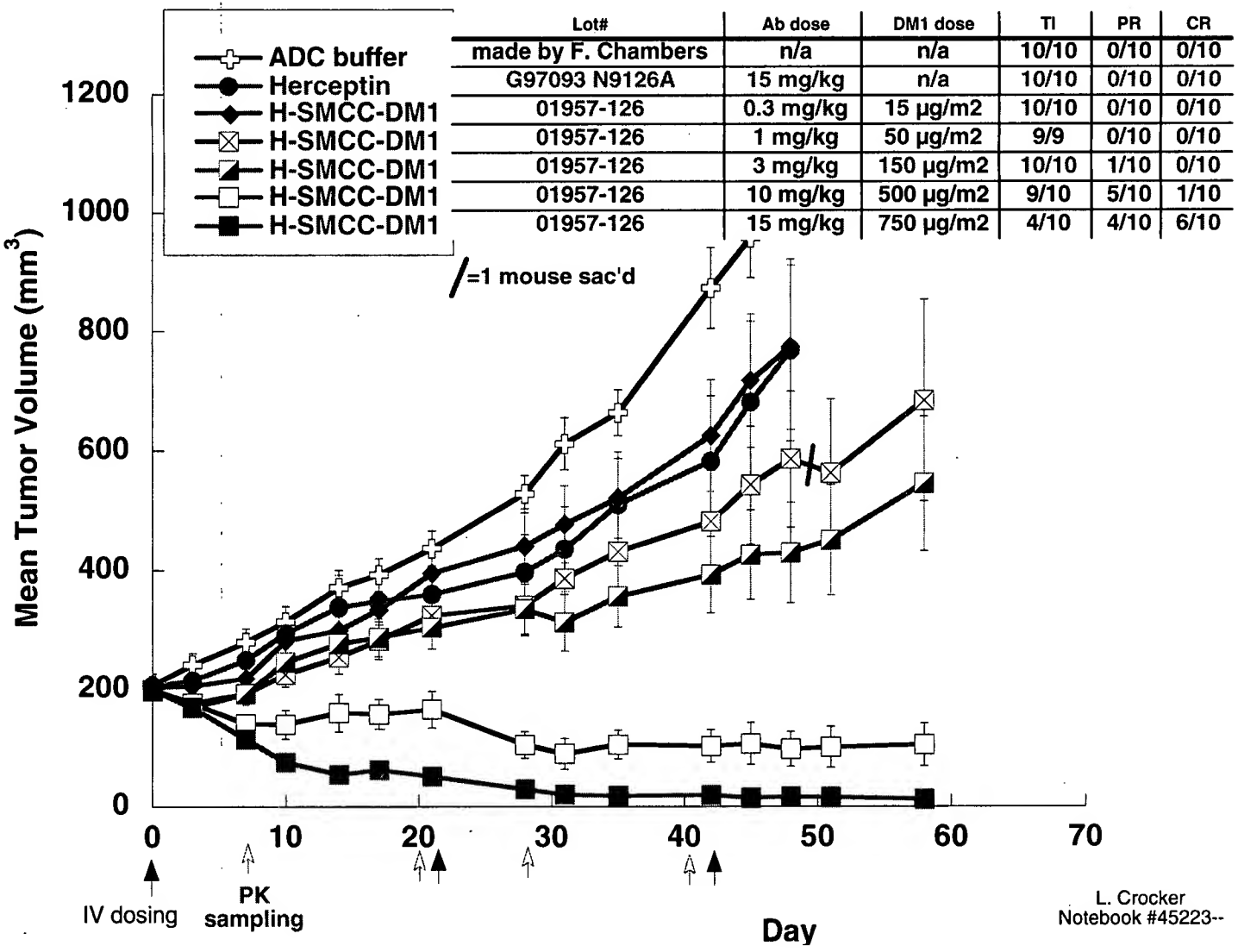


Drug	TI	PR	CR
n/a	6/6	2/9	0/9
n/a	8/9	8/9	1/9
500 µg/m2	1/9	1/9	8/9
1500 µg/m2	1/9	1/9	8/9

Appendix 2, Figure 1

04-0962: Extended Dose Response of Herceptin-SMCC-DM1 on BT474EI Xenograft Tumors in Beige Nude Mice (20 million cells (in matrigel)/mouse)

Lot#	Ab dose	DM1 dose	TI	PR	CR
made by F. Chambers	n/a	n/a	10/10	0/10	0/10
G97093 N9126A	15 mg/kg	n/a	10/10	0/10	0/10
01957-126	0.3 mg/kg	15 µg/m2	10/10	0/10	0/10
01957-126	1 mg/kg	50 µg/m2	9/9	0/10	0/10
01957-126	3 mg/kg	150 µg/m2	10/10	1/10	0/10
01957-126	10 mg/kg	500 µg/m2	9/10	5/10	1/10
01957-126	15 mg/kg	750 µg/m2	4/10	4/10	6/10



Appendix 2, Figure 2

Re: United States Patent Application No. 09/811,123
Filed: March 16, 2001
Title: Methods of Treatment Using Anti-ErbB Antibody-Maytansinoid Conjugates
Inventors: ERICKSON et al.

DECLARATION

I, Stuart Lutzker of Walnut Creek, California, do declare that:

1. I am a medical doctor with experience treating cancer patients, including breast cancer patients.

2. I have over 14 years of experience in medical oncology research and clinical oncology. I received M.D. and Ph.D. degrees from Columbia University, New York. My medical training includes an internship and residency in internal medicine at Yale University, and a medical oncology fellowship at Yale University and Robert Wood Johnson Medical School/University of Medicine and Dentistry of New Jersey.

3. I am currently employed as Associate Medical Director at Genentech, Inc. I have previously served as an Assistant Professor of Medicine at Robert Wood Johnson Medical School/University of Medicine and Dentistry of New Jersey. As the lead medical director of the HER2 drug conjugate team at Genentech, I was responsible for planning, gathering and co-coordinating the scientific work related to development and the clinical plan for HER2 drug conjugate therapeutic candidates. I am an author on over twenty published scientific and clinical articles related to my work, have published many abstracts and presented my work at numerous scientific and medical meetings.

4. I have worked with other clinicians who are engaged in treating patients in these and in related clinical areas and have observed their level of skill. Based on my education, training, and extensive experience as a medical doctor treating cancer patients, I have direct knowledge of the skills and knowledge of one of ordinary skill in the art in the fields related to antibody treatments for cancer, and to antibody-toxin conjugate treatments for cancer.

5. It is my considered opinion, based on my experience as a clinician, that the level of skill in the relevant art is very high, most medical doctors in the area of cancer treatment having advanced medical training including residency and often fellowship training and experience.

6. I have read and am familiar with the specification and pending claims of the present application. Based on my training and experience, I am familiar with scientific fields related to antibody treatments for cancer, and to antibody-drug conjugates.

7. Based on my clinical experience and knowledge, I believe that about 18-25% of breast cancer patients overexpress ErbB2 (depending on the method testing).

8. The anti-ErbB2 antibody HERCEPTIN® is indicated for patients with breast cancer that over-express HER2 or have evidence of HER2 gene amplification.

9. The clinical benefit of HERCEPTIN® treatment varies based upon the clinical setting and whether it is given as a single agent or in combination with chemotherapy. Response rates to treatment with HERCEPTIN® alone vary from 15 to 26%. HERCEPTIN® is also used in combination with other cancer treatments.

10. Analysis of randomized clinical trial data in HER2+ MBC (metastatic breast cancer) indicates that HERCEPTIN® provides improved response rate, time to progression and survival when combined with chemotherapy. In the pivotal study of HERCEPTIN® with chemotherapy (either an anthracycline or paclitaxel), the response rate increased from 32 to 50%; many patients with prolonged stable disease also benefited from HERCEPTIN® in this trial so the exact percentage of patients with benefit cannot be calculated. In a single agent study of HERCEPTIN®, 38% of patients had clinical benefit defined as either a response or stable disease for 6 months. The remaining patients gained either no or little clinical benefit from single agent HERCEPTIN® as described in U.S. Patent application 09/811,123 (at page 5, lines 14-22, for example). In addition, all patients with HER2+ MBC eventually progress despite receiving HERCEPTIN® and thus require alternative therapies.

11. Clinical experience and clinical studies now indicate the existence of at least two populations of patients whose cancers overexpress ErbB2 and progress despite HERCEPTIN® treatment: those with primary HERCEPTIN® resistance (best response to prior HERCEPTIN® being progressive disease) and those with acquired HERCEPTIN® resistance (best response having been either tumor shrinkage or prolonged stable disease). There is currently no clear mechanism for HERCEPTIN®-resistance although several have been put forward with varying degrees of supportive clinical evidence. Thus, it is not clear at this time what characteristics, other than response to treatment with HERCEPTIN® alone, distinguish the subpopulations of breast cancer patients.

12. I note, for example, a recent article (Spector et al (2005) Jour. of Clin. Onc. 23(11):2502-2512) that makes the point that HERCEPTIN® non-responding patients continue to express or overexpress ErbB2. The majority of patients entered onto this study had previously received clinical benefit from HERCEPTIN®. The observation that these patients continue to over-express HER2 was important and not expected. Prior to this recent observation, it would not have been obvious to investigate or to determine whether or not a patient fell into that subpopulation of patients. I believe that this recent observation corroborates and supports the usefulness of the methods described in U.S. Patent Application 09/811,123.

13. I believe that clinicians would find information concerning which population a patient fell into to be clinically useful. For example, in determining a treatment regimen for treating patients, I believe it could be clinically useful to know whether the patient was one whose cancer overexpressed ErbB2 yet did not respond, or responded poorly, to anti-ErbB2 antibody treatment; and that it could be clinically useful to know whether or not the patient had a cancer that overexpressed ErbB2 and did respond to anti-ErbB2 antibody treatment.

14. I believe that it would be helpful in my clinical practice to have available treatment methods to help those patients whose cancers overexpress ErbB2 yet who do not seem to be helped by anti-ErbB antibody treatment.

15. I do not believe that cancer patients with a cancer that overexpresses ErbB2 have yet been treated with anti-ErbB antibody-Maytansinoid conjugates. However, clinical studies with anti-ErbB2 antibody-Maytansinoid conjugates are scheduled to start in early 2006. This drug will be studied in patients with HER2+ metastatic breast cancer who have progressed on or shortly after stopping HERCEPTIN®. The initial studies will administer the drug every 3 weeks for an assessment of toxicity and to identify the correct dose for further studies.

16. Genentech has performed *in vitro* and *in vivo* studies to demonstrate that optimal activity of the conjugate requires high HER2 expression. Genentech has also studied the conjugate in two models of HERCEPTIN®-resistant HER2+ breast cancer and has demonstrated significant activity. These preclinical studies and the recent knowledge that tumors maintain high HER2 expression despite progressing on HERCEPTIN® provide a strong rationale for developing the conjugate in the indicated patient group. As I noted above, recent clinical studies have demonstrated that Her2 antigen continues to be over-expressed on the surface of breast cancer cells once the tumor is clinically resistant to HERCEPTIN®. I believe that this new data is important because it provides an additional rationale for treating this separate, identified patient population with the conjugate targeted to the Her2 antigen.

17. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true. I declare that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application of any patent issuing thereon.

Date: 9-1-05

Stuart Lutzker, M.D., Ph.D.
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